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The Use of *Astacus leptodactylus* (Eschscholtz, 1823) as a Test Species for Toxicity Evaluation of Municipal Wastewater Treatment Plant Effluents

Cikcikoglu Yildirim N.[1*], Aksu Ö.[2], Tatar S.[1] and Yildirim N.[1]

1. Department of Environmental Engineering, Faculty of Engineering, Munzur University, 62000 Tunceli, Turkey

2. Fisheries Faculty, Munzur University, TR62000, Tunceli, Turkey

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ABSTRACT: This study evaluated the responses of biochemical biomarkers in *Astacus leptodactylus* exposed to treated municipal effluents discharged into Keban Dam Lake, Elazığ Elazig, Turkey. *A. leptodatyclus* were exposed to treated municipal effluents and Catalase (CAT), Superoxide dismutase (SOD) activity and lipid peroxidation (TBARS), glutathione (GSH) levels were measured as oxidative stress biomarkers. SOD activity was increased after exposing to treated municipal effluents for 24th and 96th h. CAT activities were decreased from 25.29 to 14.12 nmol/min/ml compared to control in the group exposed to treated municipal effluents for 24 h but it increased after 96 h exposure. GSH levels were decreased from 9.08 to 3.77 µM compared to control, but MDA levels were increased both at 24 th h and 96 th h after exposure to treated municipal effluents. CAT, SOD activities and MDA and GSH levels in the hepatopancreas of *A. leptodactylus* are sensitive and suitable biochemical biomarkers for evaluating the toxicity of the treated municipal effluent complex mixtures. Treated municipal effluents exposure was found to cause sub-lethal responses in *A. leptodactylus* suggesting oxidative stress.

Keywords: *Astacus leptodactylus, Wastewater Effluent, Oxidative Biomarkers, Toxicity.*

INTRODUCTION

Treated municipal effluents (TME) are the major sources of aquatic pollution by numerous chemicals compounds in completely eliminated during the treatment processes (Kamel *et al.,* 2015). Different pollutants present in TME are known to cause the toxic effect and a variety of stress-related changes on non-target species at low concentration (ng/L to µg/L range) (Fatima and Ahmad 2005; Gagne *et al.,* 2008).

Chemical analysis is not sufficient to assess the water quality of wastewater (Radic *et al.,* 2011). Toxicological assessment by using model organism has gained much attention, since it provides the complete response of test organisms to all compounds in wastewater (Movahedian 2005). If the quality of TME is poor, more treatment can be needed to improve the quality of discharged effluent (Weis and Weis 2004; Brix and Arias 2005; Chen *et al.,* 2005; Upadhyay *et al.,* 2007). Using biochemical biomarkers has been demonstrated as an effective tool in the assessment of treated wastewater quality (Ghedira *et al.,* 2009).

Antioxidant biomarkers can be used as biomarkers for aquatic contamination, as various contaminants destroy the balance

* Corresponding Author Email: nurancyildirim@gmail.com, Tel:+905303649999

between pro-oxidants and antioxidants (Monserrat *et al.,* 2007). Pollutants can cause to increase reactive oxygen species (ROS). The antioxidant defense system is triggered as a compensatory response and oxidative damage occurs. Therefore the antioxidant response can be used as a non-specific biomarker of aquatic contamination (Bainy *et al.,* 1996; Geracitano *et al.,* 2004).

Elazig municipal wastewater treatment plant uses the conventional activated sludge process to treat the wastewater of Elazig. After treatment in the effluent from the city finished, treatment plant is discharged into Keban Dam Lake (Karatas *et al.,* 2009).

The freshwater crayfish, *Astacus leptodactylus*, is naturally and widely distributed in lakes, ponds and rivers throughout Turkey (Harlioglu 2013). The toxicity of municipal wastewater treatment plant effluents to narrow-clawed crayfish, *A. leptodactylus* Esch. has not been previously studied. In this study, the antioxidant response of *A. leptodactylus* exposed to acute TME was used to assess the efficiency of Elazig municipal wastewater treatment plant.

MATERIALS AND METHODS

Ten liters of waste water taken from Elazig municipal wastewater treatment plant the day before and the samples were stored at 4 ºC for the experiments. The physicochemical properties of the reference water and the treated effluent are shown in Table 1. Reference freshwater sampling point (397.3690 N 3930.9970 E) was selected according to existing pollution impact characteristics (physicochemical characteristics of water, other anthropogenic pressures). Ten liters of reference surface water samples were collected with standard equipment from the middle of the Munzur river at a depth of 10 cm.

Table 1. Some physicochemical Characteristics of TME and The Reference Water (RW).

Parametres	TME	RW
pH	7.52	7.67
Temperature ºC	12	11
Conductivity (ms/cm)	1192	396
COD (mg/L)	67.46	25.00
BOI (mg/L)	380	3.8
Total Dissolved Solid (TDS) (mg/L)	19	2.88
Dissolved Oxygen (mg/L)	8.38	6.62

The organisms were rapidly transferred to the laboratory where they were stock in aerated 20 L tanks in a climate controlled room at 18 ºC, a 12:12 light: dark cycle. And fed willow leaves for 15 d before experimental studies.

Each tanks consists of 10 L water (not diluted) consisted of 3 replicates with 10 individuals. Organisms were not fed during the experiments. The organisms were checked per 24 h. Two experimental groups were designed as: Reference water (RW) (Munzur river) and TME Exposure Group. The experiments included the investigation of the exposure of two groups of *A. leptodactylus* (n: 10 for each group) for 24 and 96 hours towards wastewater samples. The animals tested for 48 and 96 hours were kept together in tanks.

Chemical Oxygen Demand (COD) contents of TME were measured using a Hach digestion and calorimeter DR/890 with LCK314COD cuvette test. Dissolved oxygen (DO), Total Dissolved Solid (TDS), Electrical Conductivity (EC), and pH were measured by hachlange multimeter device. Biochemical Oxygen Demand (BOD_5) was measured with aquatic lytic AL606 BOD device.

The crayfishes were caught with fykenettraps from Keban Dam Lake Agin, Elazig. Crayfishes were adapted in tap water for 1 week before the experiment. The water in the tank (20 L) was aerated during the experiment. The crayfishes were fed with commercial feed during their adaptation period. After adaptation, they transported to the laboratory of Munzur university, department of environmental engineering, and aquatic toxicology laboratory. The crayfishes have exposed to TME for 24 h and 96 h.

The crayfishes were dissected and hepatopancreas tissue of them was removed. To analyze the biochemical biomarkers, the tissues were weighed. They were homogenized in 1/5 w/v PBS buffer. Then the samples were centrifuged for 15 min at 17.000 rpm. The supernatants were stored in deep freeze at −70 °C until the analysis was done.

SOD and CAT activities and GSH and lipid peroxidation levels (TBARS) were determined by using ELISA kits from CAYMAN Chemicals Company (catalog numbers, SOD: 706002, CAT: 707002, and TBARS: 10009055, GSH: 703002).
MDA–TBA adduct formed by the reaction of MDA and TBA was measured as a secondary lipid peroxidation product by using TBARS ELISA kit

The significance of differences between exposure groups and exposure times were determined using one-way ANOVA and independent t-test.

RESULTS AND DISCUSSION

Physico-chemical parameters of treated wastewater and reference water were shown in Table-1.

SOD enzyme activity was increased in the group exposed to TME compared to the control group during 24 and 96 hours but this increase was not statistically significant ($p>0.05$). Exposure time is not statistically significant ($p>0.05$) (Fig-1).

CAT activity was decreased in the group exposed to TME after 24 h but increased after 96 h compared to the control group ($p<0.05$). CAT activity was decreased in the control group at 96^{th} h compared to 24^{th} h ($p<0.05$) (Fig-1).

GSH levels were decreased after exposure to TME after 24 and 96 h compared to control ($p<0.05$). GSH levels were increased in control and TME exposure group at 96^{th} h compared to 24^{th} h ($p<0.05$) (Fig-1).

TBARS levels were increased in TME exposure group compared to control during 24 and 96 hours ($p<0.05$). TBARS level were increased in TME exposure group depending on exposure time ($p<0.05$) (Fig-1).

Municipal wastewaters are complex mixtures that contain toxic, carcinogenic and mutagenic organic or inorganic pollutants which are all difficult to determine (Cristale and Lacorte 2015). It was indicated that physico-chemical parameters alone were not sufficient in obtaining reliable information on treated wastewater toxicity (Movahedian *et al.,* 2005). The use of biomarkers as an alternative to chemical analyzes to assess the Environmental impact of wastes has received considerable interest in recent times (Petala *et al.,* 2009). In the present study, it was determined that whether *A. leptodactylus* can be a useful analytical tool for control discharges and ecological impact assessment. For this purpose, biochemical biomarkers (SOD, CAT, GSH and TBARS) in *A. leptodatylus* exposed to the TME from Elazig municipal wastewater treatment plant.

Lipid peroxidation is a free radical chain reaction (Kappus 1987). MDA, a by-product of lipid peroxidation and used as an indicator of lipid peroxidation (Cheesman and Slater 1993). It has been demonstrated that TME may cause oxidative damage in aquatic organisms via increased lipid peroxidation (Oakes and Van Der Kraak 2003; Vega-Lo´ pez *et al.,* 2008; Petala *et al.,* 2009). Thiobarbituric reactive substances (TBARS) levels have been used as an indicator of lipid peroxidation. (Valavanidis *et al.,* 2006).

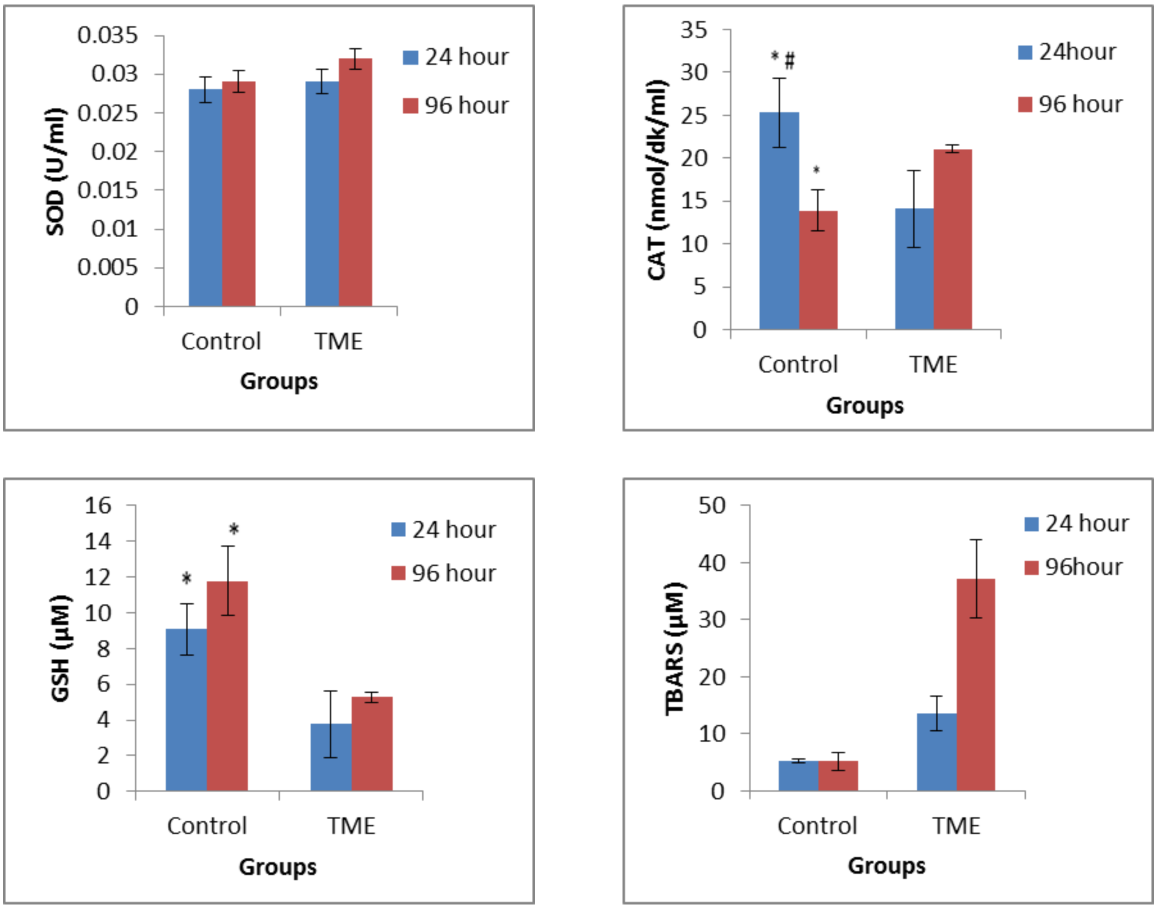

Fig. 1. SOD, CAT activities and GSH, TBARS levels in *A. leptodactylus* exposed to TME during 24 and 96 h. *Shows the difference between groups (between tap water and TME) at 24 and 96 hours. # indicates the difference between the exposure times.

Petala *et al.* (2009) found that unchlorinated, ozonated and chlorinated effluents cause to increase of TBARS due to the elevated ROS formation reflecting possible lipid peroxidation. Increased TBARS levels were found in *Ruditapes decussatus* after exposure to TME for 2-weeks (Kamel *et al.*, 2012). Barim *et al.* (2010) investigated the effects of pollution on *A. leptodatylus* and high MDA levels were found due to the wastewaters released from the Leather Factory aforementioned. Tatar *et al.* (2018) indicated increased MDA levels after exposure to TME of Tunceli wastewater treatment plant for 96 h. Tatar *et al.* (2017) also found elevated mda levels in the fronds of *Lemna minor* and *Lemna gibba* after exposure to TME from municipal wastewater treatment plant, Elazig. Díaz-Garduño *et al.*, (2018) investigated the effects of the different effluent concentration in *Solea senegalensis* after 7 days of exposure. They found that lipid peroxidation levels were decreased at 1/8 and 1/4 dilution rate. Karataş *et al.*, (2008) demonstrated that the increased MDA levels in *Lemna gibba* L. after exposure to secondary effluent. Gillis et al. (2014), and Machado et al. (2014) also observed increased lipid peroxidation levels in wild freshwater mussels collected from locations downstream of municipal wastewater effluent outfalls. In the present study, similarly lipid peroxidation levels were increased in TME exposure group compared to control during 24 and 96 hours.

GSH is related to the biotransformation of xenobiotics, which is known to be one of the first lines of the defense against the ROS (Noctor and Foyer 1998). Elevated hepatic GSH levels in rainbow trout after exposure to sewage treatment plant effluents were found (Almroth *et al.,* 2008). They suggested that increased GSH levels implying increased GSH synthesis. Aksu *et al.,* (2014) investigated the biochemical response of *A. leptodactylus* exposed to treated and untreated textile wastewater during 24 and 96 h to assess the treatment efficiency of white rot fungus (*C. versicolor*). They suggested that GST activities are increased in untreated group after treatment GST values return the control values. GSH levels were increased in *Gammarus pulex* exposed to the secondary effluent of the treatment plant after 24 and 96 h exposure (Tatar *et al.*, 2018). GSH levels were increased in rats exposed to wastewater (Tabrez and Ahmad 2009).

SOD and CAT plays a very important role in the antioxidant defense CAT catalyzes the H_2O_2 to H_2O and O_2. SOD catalyzes the dismutation of O_2 and defends the organism against the toxic effects of ROS. They can be used as an oxidative stress biomarker after exposure to xenobiotics (Almeida *et al.*, 2007; Jebali *et al.,* 2007; Prasad 2004). Overproduction of reactive oxygen species due to the environmental pollutants cause to induce CAT activity (Vioque-Ferna´ndez *et al.,* 2009). Kamel et al. (2012) suggested that exposure of clams to TME induce the elevated CAT activity in rainbow trout exposed to municipal wastewater was also found (Sturve *et al.,* 2008). It was suggested that CAT activity was increased in male rainbow darter exposed to wastewater treatment plant effluent due to the increased ROS (Mehdi *et al.,* 2017). CAT activity was decreased in *Gammarus pulex* exposed to secondary effluent (Tatar *et al.,* 2018). In our study, CAT activity was also increased after 96 h as a protective response to increased ROS. In this study, the increase in CAT activity in *A. leptodatylus* due to the increased lipid peroxidation levels. Environmental pollutants were demonstrated to induce SOD enzyme activity in many studies (Dimitrova *et al.,* 1994; McCord 1996; Dautremepuits *et al.,* 2004). Tatar *et al.* (2018) indicated that SOD activity was changed depending on exposure time in *G. pulex* exposed to secondary effluent. Increased SOD activity was observed in *Mytilus trossulus* exposed to the effluents of wastewater treatment plant (Turja *et al.,* 2015). In the present study, increased SOD enzyme activity indicated that superoxide radical levels were strongly enhanced (Prasad *et al.,* 1999).

CONCLUSION

Statistically significant changes were observed in all biomarkers of *A. leptodactylus* exposed to TME except SOD. Overall, several of biochemical biomarkers (CAT, SOD activities and MDA, GSH levels) in the hepatopancreas of *A. leptodactylus* show promise as biomarkers of the toxicity of the treated municipal effluent complex mixtures. Additionally, the conventional activated sludge process of Elazig municipal wastewater treatment plant was tested in order to reduce the adverse effects on *A. leptodactylus.* TME exposure was found to cause sub-lethal responses in *A. leptodactylus* suggesting oxidative stress.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

REFERENCES

Aksu, O., Yildirim N.C., Yildirim, N., Danabas, D. and Danabas, S. (2015). Biochemical response of crayfish *Astacus leptodactylus* exposed to textile wastewater treated by indigenous white rot fungus *Coriolus versicolor*. Environ. Sci. Pollut. Res., 22:2987–2993.

Almroth, B.C., Albertsson, E., Sturve, J. and Forlin, L. (2008). Oxidative stress, evident in antioxidant defenses and damage products, in rainbow trout caged outside a sewage treatment plant. Ecotoxicol. Environ. Saf., 70:370–378.

Bainy, A.C.D., Saito, E., Carvalho, P.S.M. and Junqueira, V.B.C. (1996). Oxidative stress in gill, erythrocytes, liver and kidney of *Nile tilapia* (*Oreochromis niloticus*) from a polluted site. Aquat. Toxicol., 34: 151–162.

Barim, Ö. and Karatepe, M. (2010). The effects of pollution on the vitamins A, E, C, β-carotene contents and oxidative stress of the freshwater crayfish, *Astacus leptodactylus.* Ecotoxicol. Environ. Saf., 73: 138-142.

Brix, H. and Arias, C.A. (2005). The use of vertical flow constructed wetlands for on- site treatment of domestic wastewater: new Danish guidelines. Ecol. Eng., 25: 491–500.

Cheesman, K.H. and Slater, T.F. (1993). Introduction to free radical biochemistry. Br. Med. Bull., 49: 481–493.

Chen, Y., Wang, C. and Wang, Z. (2005). Residues and source identification of persis- tent organic pollutants in farmland soils irrigated by effluents from biological treatment plants. Environ. Int., 31: 778–783.

Cristale, J. and Lacorte, S. (2015). PBDEs versus NBFR in wastewater treatment plants: occurrence and partitioning in water and sludge. AIMS Environ. Sci., 2: 533-546.

Dautremepuits, C., Paris-Palacios, S. and Betoulle, S. (2004). Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L) induced by copper and chitosan. Comp. Biochem. Physiol C Toxicol. Pharmacol., 137:325–33.

Diaz-Garduno, B., Perales, J.A., Biel-Maeso, M., Pintado-Herrera, M.G., Lara-Martin, P.A.,Garrido-Pérez, C. and Martín-Díaz, M.L. (2018). Biochemical responses of *Solea senegalensis* after continuous flow exposure to urban effluents. Sci. Total Environ., 615:486-497.

Dimitrova, M.S.T., Tsinova, V. and Velcheva, V. (1994). Combined effect of zinc and lead on the hepatic superoxide dismutase–catalase system in carp (*Cyprinus carpio*). Comp. Biochem. Physiol., 108:43–6.

Fatima, R.A. and Ahmad, M. (2005). Certain antioxidant enzymes of Allium cepa as biomarkers for the detection of toxic heavy metals in wastewater. Sci. Total Environ., 346: 256-273.

Gagne, F., Andre, C., Cejka, P., Hausler, R., Fournier, M. and Blaise, C. (2008). Immuno- toxic Effects on freshwater mussels of a primary-t,reated wastewater before and after ozonation:apilot plant study. Ecotoxicol. Environ. Saf., 69:366–373.

Geracitano, L.A., Monserrat, J.M. and Bianchini, A. (2004). Oxidative stress in *Laeonereis acuta* (Polychaeta, Nereididae):environmental and seasonal effects. Mar. Environ. Res., 58:625–630.

Ghedira, J., Jebali, J., Bouraoui, Z., Banni, M., Chouba, L. and Boussetta, H. (2009). Acute Effects of Chlorpyriphos-ethyland Secondary Treated Effluents on Acetylcholinesterase and Butyryl Cholinesterase Activities in *Carcinus maenas*. J. Environ. Sci., 21:1–6.

Gillis, P.L., Gagné, F., McInnis, R., Hooey, T.M., Choy, E.S., André, C., Hoque, M.E. and Metcalfe, C.D. (2014). The impact of municipal wastewater effluents on field-deployed freshwater mussels in the Grand River (ON). Environ. Toxicol. Chem., 33:134–143.

Harlioglu, M.M., Cakmak, M.N., Köprücü, K., Aksu, O., Harlioglu, A.G., Yonar, M., Duran, S.C.,Ozcan, S.T. and Gundogdu, H. (2013). The effect of dietary n-3series fatty acids on the number of pleopadal egg and stage 1 juvenile in freshwater crayfish, *Astacus leptodactylus* Eschscholtz. Aquacult. Res., 44:860–868.

Karatas, F., Öbek, E. and Kamıslı, F. (2009). Antioxidant capacity of *Lemna gibba* L. exposed to wastewater treatment, Ecol. Eng., 35:1225–1230

Kamel, N., Jebali, J., Banni, M., Khedher, S.B., Chouba, L. and Boussetta, H. (2012).Biochemical responses and metals levels in Ruditapes decussatus after exposure to treated municipal effluents, Ecotoxicol. Environ. Saf., 82:40–46.

Kappus, H.A. (1987). A survey of chemicals inducing lipid peroxidation in biologicalSystems. Chem. Phys. Lipids 45: 105–115.

Machado, A.A.S., Wood, C.M., Bianchini, A. and Gillis, P.L. (2014). Responses of biomarkers in wild freshwater mussels chronically exposed to complex contaminant mixtures. Ecotoxicology 23:1345–1358.

Mehdi, H.H., Dickson, F., Leslie, M., Bragg, M., Servos, R. and Craig, P.M. (2017). Impacts of wastewater treatment plant effluent on energetics and stress response of rainbow darter (*Etheostoma caeruleum*) in the Grand River watershed. Comp. Biochem. Physiol. A, 1096-4959:30184-7.

McCord, J.M. (1996). Effects of positive iron status at a cellular level. Nutr Rev 54:85–8

Monserrat. J,M,, Martınez. P.E., Geracitano, L.A., Amado, L.L., Martins, C.M., Pinho, G.L., Chaves, I.S., Ferreira-Cravo, M., Ventura-Lima, J. and Bianchini, A. (2007). Pollution biomarkers in estuarine animals: critical review and new perspectives. Comp. Biochem. Physiol. C, 146:221–234.

Movahedian, H., Bina, B. and Asghari, G.H. (2005). Toxicity Evaluation of Wastewater Treatment Plant Effluents Using *Daphnia magna*. Iranian J. Environ. Health Sci. Eng., 2: 1-4.

Noctor, G. and Foyer, C.H. (1998). Ascorbate and glutathione: keeping active oxygen undercontrol. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49:249–279.

Oakes, K.D. and VanDerKraak, G.J. (2003). Utility of the TBARS assay in detecting oxidative stress in whitesucker (*Catostomus commersoni*) populations exposed to Pulpmil leffluent. Aquat. Toxicol., 63,447–463.

Petala, K.M., Samaras, L., Papadopoulos, P. and Zouboulis, A. (2009). Toxicological and ecotoxic impact of secondary and tertiary treated sewage effluents. Water Res., 43:5063–5074.

Prasad, M.N.V. (2004). Heavy Metal Stress in Plants: From Biomolecules to Ecosystems, Springer, Berlin Heidelberg Prasad, K.V.S.K., Saradhi, P. and Sharmila. P, (1999), Concerted action of antioxidant enzymes and Curtailed growth under zinc toxicity in Brassica juncea. Environ. Exper. Bot., 42:1–10

Radic, S., Stipanicev, D., Cvjetko, P., Sırac, S., Pevalek-Kozlina, B. and Pavlica, M. (2011). Duckweed *Lemna minor* as a tool for testing toxicity and genotoxicity of surface waters. Ecotoxicol. Environ. Saf., 74:182–187.

Sturve, J., Almroth, B.C. and Förlin, L. (2008). Oxidative stress in rainbow trout (*Oncorhynchus mykiss*) exposed to sewage treatment plant effluent. Ecotoxicol. Environ. Saf., 70: 446–452.

Upadhyay, A.R., Virendra, K., Mishra, V.K., Sudhir, K., Pandey, S.K. and Tripathi, B.D., (2007). Biofiltration of secondary treated municipal wastewater in a tropical city. Ecol. Eng., 30:9–15.

Tabrez, S. and Ahmad, M. (2009). Effect of wastewater intake on antioxidant and marker enzymes of tissue damage in rat tissues: Implications for the use of biochemical markers. Food Chem. Toxicol., 47:2465–78.

Tatar, S., Yildirim, N.C., Serdar, O., Yildirim, N. and Ogedey, A. (2018). The using of *Gammarus pulex* as a biomonitor in ecological risk assessment of secondary effluent from municipal wastewater treatment plant in Tunceli, Turkey. Hum. Ecol. Risk Assess, 24(3):819-829.

Tatar, S., Obek, E. and Yildirim, N.C. (2017). Antioxidant Response in Duckweed After Exposure to Secondary Effluent from Municipal Wastewater Treatment Plant, Elazığ, Turkey. Bull. Environ. Contam. Toxicol., 99:399-404.

Valavanidis, A., Vlahogianni, T., Dassenakis, M. and Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf., 64(2):178–189.

Vega-Lopez, A., Jimenez-Orozco, F.A., Garcia-Latorrec, E. and Dominguez-Lo´pez, M. (2008). Oxidative stress response in an endangered goodeidfish (*Girardinichthys viviparus*) by exposure to water from its extantlocalities. Ecotoxicol. Environ. Saf., 71:94–103.

Vioque-Fernandez, A., AlvesdeAlmeida, E. and Lo´pez-Barea, J. (2009). Assessment of Don˜ana National Park contamination in Procambarusclarkii: integration of conventional biomarkers and proteomic approaches. Sci. Total Environ., 407:1784–1797

Weis, J.S. and Weis, P. (2004). Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ. Int., 30:685–700.